CHAPTER 4

Immunology and Immunotherapy of Intrinsic Glial Tumors

William T. Couldwell, MD, Malcolm S. Mitchell, MD,
Amitabha Mazumder, MD, and Michael L.J. Apuzzo, MD

Immunological aspects of glial neoplasms have been actively investigated for the past several years with particular focus on academic, diagnostic, and therapeutic objectives. As more knowledge is acquired about the cellular events relating to tumor behavior the more it appears that the immune system is a pivotal tool for tumor evaluation. Conceivably, it may prevail as the ultimate resource in combating tumor growth. In this light, it behooves all neurosurgeons to understand the basic functional concepts of the immunological system and its relevance to neoplastic growth. Current standard therapies have surely done little to improve the poor prognosis manifest in patients with malignant glioma.[74]

The Normal Immunological Response

The normal immune system has three basic cellular elements: the T-cell, the macrophage, and the B-cell. The T-cell and the B-cell make up the lymphocyte group, characterized by a size ranging from 4-20 mm (small and large) and a large nucleus staining deep blue with Wright's stain. Subsets of lymphocytes may be distinguished by molecular configuration of the cell surface membranes. Cells defined as B-cells (Bursa-derived, in the chicken) are characterized by immunoglobulin on the cell membrane. These cells are primarily concerned with the production of circulating immunoglobulin and thus comprise the humoral arm of the immune response. T-cells (thymus derived) have the so-called theta-antigen on their surface in mice, and in man, the CD3 surface antigen. They also possess a specific receptor for antigen—analogous to, but not identical with, the immunoglobulin situated on B-cells. In the peripheral lymph nodes they reside primarily in the deep subcortical areas and around the germinal centers. These cells are primarily involved in cell-mediated immunity (Figure 1).

Cellular Immune Response

The so-called cell-mediated immune response is foremost in consideration of antitumor immunity. When sensitized T-cells and macrophages are activated, they are capable of sensitizing and killing tumor cells, a function that probably occurs with an expected statistical frequency in the normal host. The thymus-derived T-cell originates in the bone marrow and achieves immunocompetence through maturation in the thymus. T-cells reside in all lymphatic organs of the body and are stimulated in the immune response by macrophages that process and present antigen, thereby enabling initiation of T-cell activation (Figure 2). In this manner the macrophage is the central component in

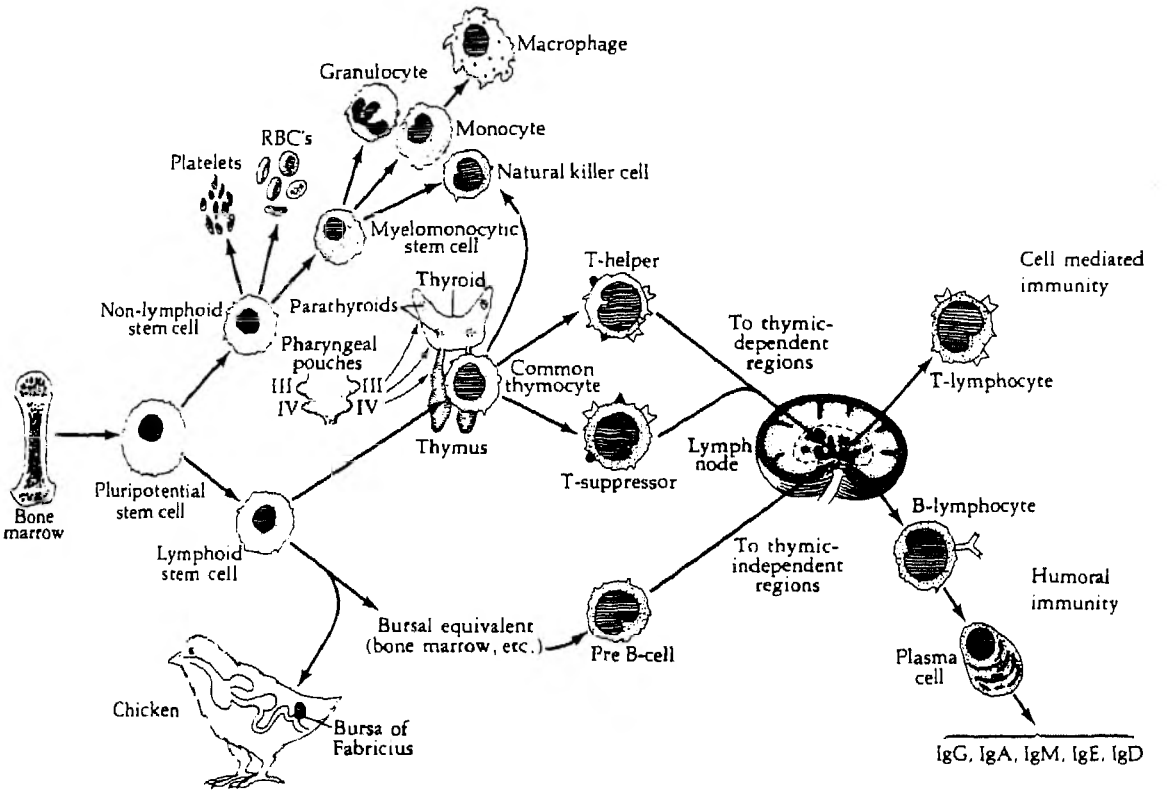

Figure 1. Development of the lymphoid system. Representation of the ontogeny of the immune response, showing differentiation of progenitor cells into hemopoietic and immunocompetent cells.

With permission from Bellanti JA. *Immunology III*. Philadelphia, Pa: WB Saunders; 1985.

both the recognition of antigen and initiation of the immune response.

As the T-cell enters its functional or effector phase, it is capable of damaging or destroying tumor cells. This function is dependent upon the release of lymphokines, soluble mediators that amplify the immune response. Lymphokines are humoral factors that influence the function of other effector cells. One lymphokine, interleukin-2 (IL-2), induces lymphocyte growth, proliferation, and differentiation.[16] Several other lymphokines have been characterized and will be discussed in detail later (Table 1).

The T-cells themselves are a heterogeneous group, encompassing several phenotypically different cell types. Antigen-driven lymphocyte differentiation of T-cells alters the display of surface macromolecules. These so-called differentiation antigens have become useful as markers of cell function. Several subclasses of T-lymphocytes are recognized, based on expression of these differentiation antigens: the CD8 positive cytotoxic (killer) effector cell; the helper T-cell, with surface expression of the CD4 antigen; and the suppressor T-cell, also with surface expression of the CD8 (formerly T5 or T8) antigen.[59] The helper T-cell aids in both cell-mediated immunity and antibody production; normal immune response to a tumor cell is dependent upon the presence of an adequate number and function of this subset of lymphocytes. Helper T-cells liberate important cytokines such as the lymphokines described above. The suppressor T-cell regulates immune response to antigens, including tumor antigens.[23] Suppressor T-cells, found predominately in the thymus and spleen within 24 hours of antigen exposure, appear to limit the

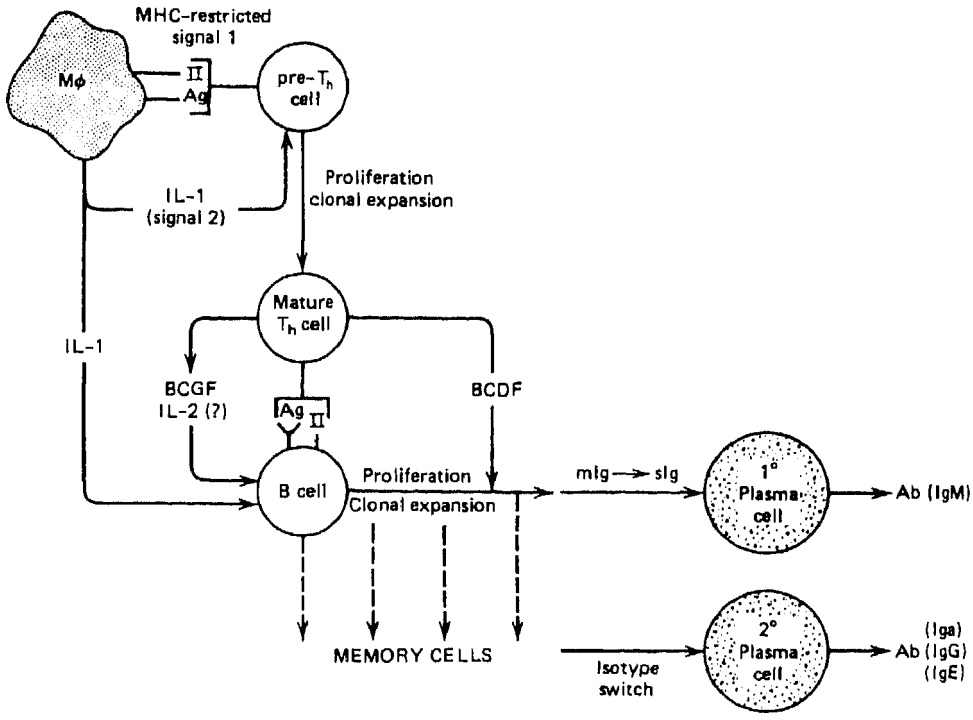

Figure 2. Cellular interactions in the production of antibody; note the central role of the macrophage (MØ). IL-2-Interleukin 2, T_h-helper T-cell.

With permission from Clark WR. *The Experimental Foundations of Modern Immunology*. New York, NY: John Wiley and Sons; 1986.

cytolytic tumor response of the host (Figure 3). Measurements of these surface markers in peripheral blood lymphocytes may offer insight in the overall immune status of the individual.

Natural killer (NK) cells are predominantly non-T lymphocytes that possess inherent cytotoxic properties to prevent the development of tumor cells into full-fledged neoplasms. These cells have the capability of lysing tumor cells in vitro without prior sensitization.[26] Their significance in glial neoplasms in vivo is an active area of investigation.

Macrophages, as emphasized above, are an integral element in the normal immune response. They are involved in almost every facet of the response.[54] The circulating monocyte, the progenitor of the tissue-residing macrophage, is derived from the same immediate precursor as the granulocyte. Blood monocytes arise from precursors in the bone marrow (Figure 1). Monocytes and macrophages represent a family of related phagocytic cells found in various lymphoid organs (spleen, liver, lymph nodes, bone marrow) and a variety of body fluids.[58] The macrophage serves a processing role for antigen, presenting antigen to T-cells, and collaborates with T-cells in tumor destruction. Macrophages also release interleukin-1 (IL-1), formerly called lymphocyte activating factor (LAF), which causes proliferation of T-cells. Chemotactic factors liberated by T-cells attract macrophages to the source of the inflammatory response; subsequently these macrophages are immobilized in the vicinity by macrophage-inhibitory factor (MIF), and thereafter activated in the presence of the antigen by macrophage-activating factor (MAF or interferon), which is probably the same as MIF.[3] The end result is a cytotoxic effector cell, capable of killing the antigen or tumor cell.

TABLE 1
Products of Activated Lympocytes*

Mediators Affecting Macrophages:
Macrophage inhibitory factor (MIF)
Macrophage activating factor (interferon-γ)
Macrophage aggregation factor
Chemotactic factor for macrophages

Mediators Affecting Neurophils:
Chemotactic factor
Leukocyte inhibitory factor

Mediators Affecting Lymphocytes:
Mitogenic factors (IL-2)
Antibody enhancing factors
Antibody suppressing factors
Chemotactic factor

Mediators Affecting Eosinophils:
Chemotactic factor
Migration stimulation factor

Mediators Affecting Basophils:
Chemotactic augmentation factor
Histamine releasing factor
Interleukin-3

Immunoglobulin Binding Factor

Procoagulant Activity

Interferon

Immunoglobulin

*Adapted from Bellanti J, Rocklin R. *Immunology III.* Philadelphia, Pa: WB Saunders; 1985.

Humoral Immune Response

The humoral immune response is the second major arm of the immune system and encompasses the stimulation, production, and liberation of antibodies to specific antigens. This charge is undertaken by the B-lymphocyte. This cell, after originating and presumably attaining immunocompetence in the bone marrow, resides in the germinal centers of peripheral lymphoid sites, ultimately maturing to become the antibody-secreting plasma cell.[75] Five major classes of immunoglobulins are recognized: IgG, IgA, IgM, IgD, and IgE. These are differentiable on the basis of the heavy peptide chain characteristics, but also on other parameters, such as their presence in external secretions and ability to traverse the placenta.

Circulating antibodies reacting with tumor-related antigens are detectable in many tumor systems.[67,79] The immunoglobulins are predominately IgG and IgM. In the presence of complement, IgM antibodies may be cytotoxic in vitro to tumor cells. IgG, on the other hand, appears to mediate cytotoxicity by facilitating such cells as macrophages or natural killer (NK) cells to recognize and destroy the tumor target. This antibody-dependent cell cytotoxicity (ADCC) is independent of complement. In comparison with cell-mediated immunity, such antibody cytotoxicity is probably of secondary importance in the defense against solid tumors.

The Immunological Response in Intrinsic Glioma

Immunological Privilege

Milestone work of Medawar[45] demonstrated the lack of immune rejection of a foreign skin graft transplanted to the brain of an unrelated individual, as compared to rejection by the skin recipient site in the same individual. Interest grew regarding the concept of the brain as an immunologically privileged site. Rationale for the concept was supported by the well-known absence of cerebral lymphatic drainage and the presence of the blood-brain barrier. Such ideas have further been substantiated by the work of Murphy and Sturm,[51] Green,[29] and others.[31,49]

Much data have since been presented to refute the concept. Neoplastic processes demonstrate permeability of the blood-brain barrier during radioisotopic brain scanning and electron microscopic examination.[66] In addition, brain structure enhancement following iodinated contrast use in computerized tomography (CT) and more recently gadolinium-DTPA in magnetic resonance imaging (MRI) is a manifestation of blood-brain barrier compromise in glioma patients. Cellular infiltrates in glioma (see page 46) is evidence that effector cells of systemic origin

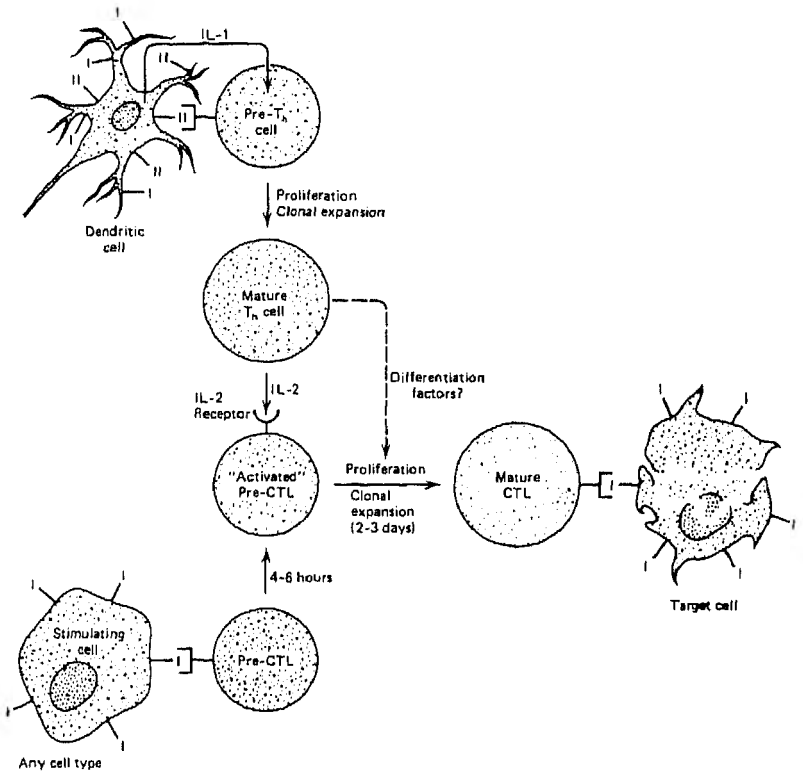

Figure 3. Cell interactions in the generation of cytotoxic T-lyphocytes (CTL). A CTL requires at least two signals for functional maturity. The first signal is a class I antigen on the allogeneic (or tumor) cell, the second signal, an activating lymphokine (e.g. interleukin-2) supplied by the helper T-cell. The helper T-cell itself requires appropriate stimulation for differentiation from an allogeneic dendritic cell bearing appropriate class II antigen on its cell surface.

With permission from Clark WR. *The Experimental Foundations of Modern Immunology*. New York, NY: John Wiley and Sons; 1986.

can enter the brain. Experimental allergic encephalitis (EAE) can be induced directly by the passive transfer of cells.[57]

Therefore, the premise of the brain as an anatomic site of partial privilege is more appropriate. It appears that the disruption of integrity of the blood-brain barrier, through neoplastic, traumatic, or inflammatory processes facilitates this loss of immune privilege.

General Immunocompetence in the Patient Harboring Glioma

Immunological reactivity is altered in patients harboring malignancy.[25] They exhibit a reduced capacity to develop delayed cutaneous hypersensitivity reactions to microbial antigens,[10] and to develop sensitivity to new antigens such as dinitrochlorobenzene.[14] Broad suppression of host immunocompetence occurs in patients harboring primary malignant brain lesions.[13] The defect is attributable, at least in part, to an impairment in T-cell function. These alterations may be the result of impaired activation processes necessary for successful T-cell mediated cytotoxicity, or quantitative and/or qualitative defects in T-cell subpopulations.[24]

Roszman et al[64] have shown the inferior production of interleukin-2 in lymphocytes of patients harboring malignant gliomas, suggesting that T-helper cell function in these patients may be suspect. This decreased ability for the T-cell to aid in the production

of antibody from B-cells has been demonstrated in vitro. An actual 25-30% diminution in the number of circulating T-helper cells in these patients has been shown. Suppressor cell function in these patients is not in itself increased; thus, the mechanism for this immunosuppression is not directly related to T-suppressor influences on T-helper cells.[62]

While much evidence supports the decreased number and function of the T-helper subpopulation of lymphocytes in patients harboring gliomas, the exact mechanism of this dysfunction and the possibility of any future therapeutic intervention are yet to be determined. One of the central issues in immunotherapy today is the pursuit of agents to stimulate the immune system in an attempt to overcome this inherent immunodeficiency in patients harboring glioma.

Cellular Infiltration in Glioma

The presence of lymphoid cellular infiltrates associated with gliomas was first appreciated by Bertrand and Mannen in 1960[5] and subsequently in 1971 by Ridley and Cavanaugh,[60] who hypothesized the significance of such infiltrates as being the manifestation of a possible host-mediated immune response to the CNS tumor. Mononuclear infiltrates have been demonstrated in the parenchyma of glial tumors in 30-60% of patients cited in the literature.[55,72] Von Hanwher et al,[72] using monoclonal antibodies and immunoperoxidase methodologies, demonstrated an in situ identification and characterization of lymphocytes within mononuclear cell populations infiltrating both human glioma and nonglial primary CNS neoplasms. This study indicated a tentative predominance of suppressor/cytotoxic ($CD8^+$) lymphoid subpopulations invading the tumor parenchyma. However, it is only assumed that the patterns of heterogeneous phenotypic lymphoid expression revealed in this study would approximate the distribution of various lymphoid subpopulations.

Good experimental evidence demonstrates that tumor-infiltrating T-cells play an integral role in the regulation of inoculated syngeneic tumor cell growth.[70] Despite this, the precise mechanisms by which T-cells are attracted to the tumor and their activity controlled are yet to be understood. As previously described, chemotactic factors for lymphocytes are soluble factors that have been isolated from sites of infiltration of T-cells. One such entity, lymphocyte migration factor (LMF), has been studied kinetically in a gliosarcoma rat tumor model and has been shown to correlate to the amount of T-cell infiltrate observed in the tumor, suggesting that at least some of the normal modulators of the immune response are present and functional in the neoplastic tissue.[77] A substantial body of literature presents conflicting insights regarding the relationship of this mononuclear infiltrate to the clinical course of glial tumors: malignant behavior, tumor burden, histological cell types, and patient survival.[12,69]

The significance of NK infiltrates in gliomas remains an enigma. While there is abundant information about the phenotypic characteristics of the lymphocytes infiltrating gliomas, none is focused on the NK cell. Recently, Vaquero et al[71] demonostrated NK cells in at least 40% of the glioblastomas in their study group of 25 patients. However, the mere presence of NK cells in the tumor did not seem to correlate with increased survival time. The NK cell, with its inherent antitumor capability, is a candidate for immunotherapeutic manipulation.

Suppressive Factors

Defective cell-mediated immunity at peripheral sites and in the tumor itself has been attributed, at least in part, to the production of immunosuppressive peptides by the neoplastic elements. The supernatant of glioma cultured cell lines exhibits suppression of thymocyte proliferation and interleukin-2-induced growth of T-cell clones. Autologous as well as homologous lymphocyte activity is

suppressible by sera obtained from these patients.[38] In patients followed postoperatively after radical tumor resection the suppressor activity is lost, only to reappear prior to clinical recurrence.[11] Roszman et al[63] observed that this glial-derived suppressive factor is effective only during the initial events of lymphocyte activation. The 25,000 molecular weight (MW) factor seems to suppress T-cell-cycling capability following stimulation. Hypothetically, this impaired immune function may be attributable to alterations in the molecular events in normal lymphocyte differentiation and growth. The factor appears to differ from the larger glycosaminoglycan moiety previously described by Gately et al,[27] which appears to function by protecting the tumor cell from cell-mediated cytotoxicity.

Wrann et al[76] recently purified a glioblastoma-derived T-cell suppressive factor (G-TsF) that exhibits suppressive effects on interleukin-2-dependent T-cell growth. This factor subsequently has been sequenced and found to have considerable homology with transforming growth factor β (TGF-β).[19] TGF-β has been demonstrated to retard the growth of a variety of transformed and normal cells and also to inhibit the generation of cytotoxic lymphocytes (CTL) and activated NK cells. Thus, it appears that several of these peptides belong to a protein family that exhibits a wide biological activity controlling the differentiation and growth of various cell types. It naturally follows that the glial tumor cells possess capabilities of expressing a wide variety of immunomodulatory substances.

Yoshida et al[80] demonstrated the qualitative defect in peripheral blood lymphocytes of patients harboring malignant brain tumors (gliomas and metastatic lesions) that inhibits capacity to produce interleukin-2. In some of their patients, the defects in interleukin-2 activity were correlated to clinical stage. This study has suggested that the immunodeficiencies seen in such patients may be at least partially attributable to the impaired production of interluekin-2. When exposed to sufficient amounts of interleukin-2, these peripheral blood lymphocytes were induced to exhibit cytotoxicity to glioma tumor cells.

Clinical Application of Monoclonal Antibodies

One approach to using immune system capabilities against glioma is to use diagnostically a specific glioma-associated antigen to differentiate the tumor from normal tissue and then to mount a specific therapeutic immune response to the antigen. Initially, polyclonal sera were used to search for tumor antigens; these sera were developed from either patients harboring gliomas or from animals inoculated with glial tumors.[17,21] Polyclonal antisera were cumbersome to employ, requiring extensive adsorption to remove nontumor-related antibodies. The advent of monoclonal antibodies (MAB) produced by the hybridoma technique, established by Köhler and Milstein in 1975,[36] enabled the production of a large quantity of antibody that will react with one specifically defined antigenic determinant. This allows identification and characterization of antigens present on human neoplastic cells. The technique requires the fusion of a specific antibody-secreting spleen B-cell with a neoplastic nonspecific antibody-secreting myeloma cell. The result is an immortal cell line with the capacity to produce unlimited quantities of a specific antibody (Figure 4).

Tumor-Associated Antigens

If such an antibody can detect an antigen that is produced exclusively or predominately by the glial tumor, the technique offers the possible use of a screening test such as carcinoembryonic antigen (CEA) to detect colon cancer. The limitation of this particular antigen, however, has been its presence in numerous other tumor locations and in some normal subjects. An analogous problem has been apparent in the search for antigens on glioma cells. To date no specific antigen

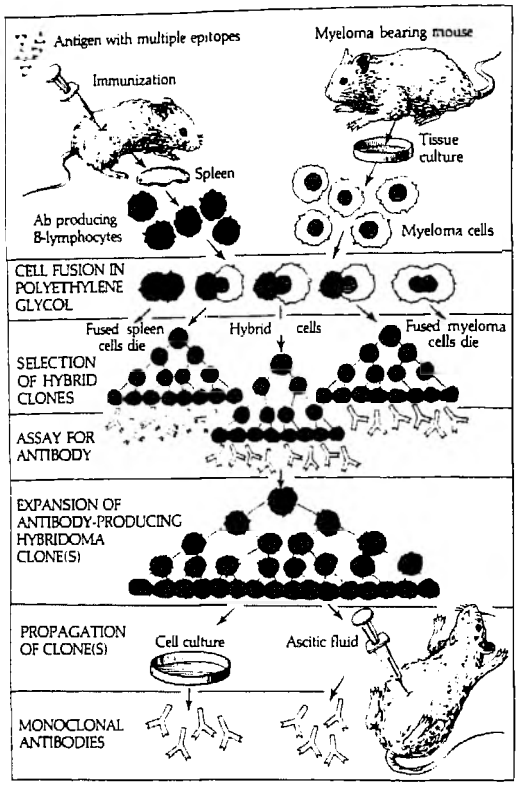

Figure 4. Schematic representation of the hybridoma technique for the production of monoclonal antibodies.

With permission from Bellanti JA. *Immunology III*. Philadelphia, Pa: WB Saunders, 1985.

unique to glioma cells has been isolated; however, several glioma antigens have been recognized, often with primary representation on glioma cells but with minor representation on other cellular constituents.[20] To further complicate the issue, cells of brain tumors have a constantly varying multiprobable antigenicity[21]; cellular antigen expression varies with cell age, attempted therapy, and heterogeneity of the original cell clonogene.

Using specific monoclonal antibodies, two groups of antigens have been identified: the neuroectodermal or neurohematopoietic antigens found on the cell surface, and glial cytoplasmic antigens such as glial fibrillary acidic protein (GFAP).[44,78] Neural differentiation antigens, such as neuroectodermal-oncofetal or neurohematopoietic-shared antigens have been detected with MAB.[79] Wakabayashi, et al[73] identified a MAB (G-22) that recognizes a neuroectodermal antigen expressed on the surface of neoplastic cells such as glioma, melanoma, and lung cancer. The further characterization of this recognized antigen proved to be a membrane-associated molecule with a molecular weight of 67 kilodaltons (KD). The antigen was also identified from CSF isolates, presumably being shed by the neoplastic cells. While the antigen is expressed on normal fetal brain tissue (eight weeks' gestation), it is not expressed by nongliomatous brain tumor of normal adult brain tissue. Thus, the presence of this reactivity in CSF or adult brain tumor tissue by means of dot-blot ELISA technique is a potential method of differentiating glioma from nongliomatous tumor tissue.

The proliferative potential of gliomas has been a problematic factor in determining the most appropriate approach to therapy, especially in the instance of low-grade lesions. Techniques such as ^{3}H-labeled thymidine autoradiography and bromodeoxyuridine (BUdR) immunohistochemical labeling have been employed to estimate the kinetic activity of the tumor as a reflection of its in vivo proliferation. Disadvantages to these methods include the necessity to administer the label prior to surgical resection. Monoclonal antibodies have been used to identify a nuclear antigen expressed specifically in proliferating cells. Ki-67 is such an antigen, which was initially raised against Reed-Sternberg cells.[28] The nuclear antigen it recognizes is present only in the G_1, S, G_2 and M phases of the cell cycle. Zuber et al,[83] studying frozen glioma specimens, demonstrated that the Ki-67 index of proliferating cells correlated with the usual histological classification of these tumors (i.e. based on the classical morphological criteria such as nuclear morphology, vascular proliferation, necrosis, and mitotic index). A significant difference was shown between Ki-67 cell index of anaplastic astrocytoma and low-grade or benign glioma. It may hold promise as a readily available and simple biochemical technique for determining this important clinical distinction.

Immunotherapy of Gliomas

Unlike surgery, radiotherapy, or chemotherapy, which have potentially toxic, limiting side effects on normal tissue, immunotherapy is inherently more specific in its selective killing of tumor cells.

The effectiveness of the immune system in reducing tumor burden may be compromised by the presence of soluble tumor antigen, antigen-antibody complexes, and nonspecific suppressor humoral substances released by the tumor. In this context, the prime theoretical directives of immunotherapy are (1) activation of antitumor cell-mediated responses; (2) activation of humoral cytolytic responses; and (3) mitigation of cellular suppressor responses.

One central theme attendant to the discussion of immunotherapy is that of tumor burden. Laboratory and clinical data have emphasized the limited ability of the normal immune system to handle excessive tumor load; a natural corollary to this is that cytoreductive measures may be an adjuvant to immunotherapy in reducing the critical neoplastic mass.

Applications of immunotherapy to glioma treatment may be categorized according to accepted immunotherapeutic treatment modes: active non-specific, active specific, adoptive, passive, and immunorestorative. With the exception of passive immunotherapy, all have been, or presently are being, evaluated for use in the treatment of glioma.

Active Nonspecific Immunotherapy

This methodology uses antigenic stimulants that are not necessarily similar to tumor antigen but increase the general immune capacity of the host. These include a variety of microorganisms such as the bacillus Calmette-Guérin (BCG)[48] and *Corynebacterium parvum*,[4] chemical agents such as pyran, and interferon inducers such as polyadenylic-uradilic acid (poly AU) and polyinosinic-cytidilic acid (poly IC). In the laboratory these agents have the ability to mitigate tumor growth and abort growth of small established tumors. They boost both cell-mediated and humoral immune responsiveness; they appear to reverse, at least in part, the suppressive effects of antitumor agents. One caveat in their usage, however, is their ability to potentiate the influence of suppressor T-cells and suppressor macrophages on the immune system, thereby limiting the overall benefit of therapy. In addition, toxic systemic and local side effects may limit effectiveness.

A number of biological response modifiers have emerged as potential agents for use in patients harboring malignancy. Lymphokines, as described above, have been advocated in this regard; interferons have been administered in the hope of stimulating the immune system to respond to antigenic tumor challenge.

Interferons are subdivided into virally induced, such as leukocyte-derived interferon (alpha), fibroblast-derived (beta), and immune interferon (gamma). Some produced by recombinant-DNA technology are under clinical scrutiny. Interferon-β, the first to be studied in glioma, has the capacity to inhibit replication of human glioma cells in vitro.[37,40] Nagai and Arai[52] treated 20 patients intrathecally or intratumorally and reported favorable results in 8 of this series. Other authors, such as Duff et al,[22] reported less enthusiastic results. Interferon-α also decreases the growth of human glioma in vitro.[53] Mahaley et al[42] described a positive response in 7 of 17 patients treated with interferon-α either intravenously or intramuscularly. More recently, Mahaley et al[41] reported their initial experience with interferon-γ. In 14 patients treated, positive response was seen definitely in only one patient, and stabilization for a period of 12 to 86 weeks was seen in an additional three. Toxicities associated with its use included hypotension during infusion, chills, fever, nausea, vomiting, and elevated liver enzymes. This preliminary series suggests that

further research should preferentially focus on the use of interferon-α and -β.

Active Specific Immunotherapy

Active specific immunotherapy refers to the use of specific immunization using substances that are antigenically related to products of the tumor. A specific antigen, such as treated tumor cells from the patient, or tumor cells from an antigenically similar tumor in another patient, may be employed to induce an immune response in the individual. Antigenicity of tumor cells may be enhanced by removing the sialic acid coating around the cells with neuraminidase. Tumor cells, killed by either x-ray or chemical agents such as mitomycin-C, have also been used to prepare cell-free antigen extracts. In this fashion, specific immunization is stimulated to combat the antigen (in this case, tumor cell). Cross-reacting antigens, either viral or bacterial, may be applied for the same functional response. The inherent pitfall in this technique is the induction of an autoimmune response to normal brain by cross-reactivity with commonly shared cellular antigens. This experimental allergic encephalitis (EAE) is a risk to be considered.

The concept is not novel. In 1960, W.H. Bloom et al[7] attempted to induce an immune response in a patient harboring a glioma by the subcutaneous implantation of glioma cells into the thighs. The patient subsequently died with pathological evidence of glioma in the brain with no evidence of immune response; in addition, tumoral spread in the thigh and regional lymph nodes was evident at autopsy.

In 1973, H.J.G. Bloom et al[6] used irradiated autologous tumor cells in patients with malignant gliomas treated by radical surgery and postoperative radiation. The results were disappointing; there was no statistical difference between patients immunized with the autologous tumor cells and those receiving surgery and radiation therapy alone. In the majority of cases in this study there was clinical evidence of tumor recurrence, proven histologically in 9 of 10 patients at autopsy. In addition, of the patients who received multiple injections of irradiated autologous tumor cells, none showed evidence of positive intradermal skin tests to indicate a successful immune response had been mounted. One plausible explanation for these disappointing results was the possibility that treatment of the cells with 15,000 rads of radiation reduced their antigenicity.

Adoptive Immunotherapy

Adoptive immunotherapy is a process whereby immunity is transferred via lymphoid cells or subcellular information. Initially, this was accomplished by the transfer of histocompatible lymphocytes from one patient to another. These carefully matched lymphocytes have prevented tumor growth in experimental systems. Subsequently, autologous peripheral blood lymphocytes were utilized as the donor source, obviating the problem of histocompatibility.

In 1972, Takakura et al[68] first reported using allogenic bone marrow cell transfusion or local intratumoral infusion of autologous or allogenic peripheral blood lymphocytes after surgical, radiation, or chemotherapeutic treatment of patients with malignant neural tumors. Lengthy survival times were reported anecdotally; this may have reflected the individual tumor's natural history. As no controls were included in the study design, the study results were difficult to interpret.

Subsequently, Young et al[81] directly inoculated autologous lymphocytes into recurrent glioblastoma tumor beds via indwelling catheters or at craniotomy. These autologous lymphocytes were isolated from peripheral blood and placed in direct contact with the tumor cells in an attempt to induce in vitro sensitization. All of the patients in the series had failed conventional therapies. Again, some remarkable anecdotal extended survivals were noted among the 17 patients treated. No treatment-related toxicity was noted.

The amalgam of cellular immunology and recombinant DNA technology has enabled

the use of recombinant lymphokines such as interleukin-2 to expand selective lymphoid populations in vitro.

In the 1980s, interest was rekindled in the use of immunotherapy with the description of a new class of lymphoid killer cell capable of killing tumor cells in vitro.[30] While attempting to isolate lymphoid cells with antitumor reactivity from tumor-bearing hosts, the incubation of lymphocytes in interleukin-2 resulted in the generation of activated lymphoid cells capable of lysing fresh, autologous, syngeneic, or allogenic tumor cells but not fresh normal cells.[82] These lymphokine-activated killer (LAK) cells are the result of incubation of peripheral blood lymphocytes with high doses of interluekin-2 for a period of three days or longer.

LAK cells are phenotypically different from classic cytotoxic T-lymphocytes; most LAK cells precursors have the surface markers Leu $4^{-}11^{+}15^{+}$.[65] Functional analysis reveals that they differ from NK cells in their ability to kill fresh tumor cells that are unaffected by the NK cells in major histocompatibility complex (MHC) unrestricted fashion. Because large amounts of these recombinant lymphokines can be produced, immunological reactions can be manipulated in vivo in ways not previously possible.[61]

Initially, Mazumder and Rosenberg[43] had demonstrated that systemic administration of interleukin-2 and lymphocytes activated by IL-2 produced evidence of pulmonary tumor metastatic regression. However, a dose-limiting toxicity of intravenously administered IL-2 was observed by Lotze et al,[39] consisting of fever, malaise, anorexia, gastrointestinal symptoms, and weight gain thought to be secondary to extravascular fluid accumulation. Mitchell et al[47] demonstrated that LAK cells may be generated in man after administration of minimally toxic doses of interleukin-2. It appears that the immunomodulatory effects of interleukin-2, at least in vitro, are very much dependent upon the dosage of the lymphokine exposed to the peripheral blood lymphoctyes. Kedar et al[34] reported that high-dose IL-2 may elicit immunosuppression mediated by lymphokine-activated killer cells and nonspecific suppressor cells.

Interest has developed in the potential benefit of autologous cellular infusions intrathecally in the hope of stimulating a direct, intense local immune response.

The killing of glioma cells by LAK cells in vitro was demonstrated elegantly by the work of Jacobs et al.[32] Subsequently this group published preliminary results of a phase 1 clinical trial in which nine patients with malignant glioma were treated with interleukin-2 or LAK cells.[33] There were no signs of systemic or neural toxicity following treatment, indicating that these elements may be safely administered to patients with malignant glioma.

The suppressive effect of local glioma supernatant has the capacity to limit the functional response of interleukin-2 activated lymphocytes; thus, intuitively, the methodology of activating LAK cells in vitro should circumvent this factor. In point of fact, when Bosnes and Hirschberg[8] compared the activity of LAK cells obtained from patients with glioma and normal subjects, they noted similar levels of cytotoxicity in the cells from the two groups. This was a surprising finding, considering the compromised status of cellular immunity in glioma patients. There was, however, a marked reduction in the number of circulating mononuclear cells, a finding supported by other investigators. Also, concurrent administration of steroids, frequently used in glioma patients, limits the potential yield of LAK cells.[56] They concluded that although LAK cells derived from a patient with glioma are highly cytotoxic, the low yield of LAK cells from these patients may limit efficacy, and that further refinement of harvest techniques should be achieved.

A long-term follow-up of a series of 24 patients with recurrent malignant glioma treated with recombinant IL-2 (rIL-2) and LAK cells was carried out by Merchant et al.[46] The patients all had glioblastoma, with the exception of one patient harboring a

high-grade oligodendroglioma. All patients had intraparenchymal injections of the LAK cells with rIL-2 into the tissue surrounding the tumor cavity. For a period of three days, all patients underwent subsequent daily injections of rIL-2 into the tumor cavity via an Ommaya reservoir or through the scalp flap. They then received an additional course of intracavitary LAK and rIL-2 10 days postoperatively. Patients all experienced increased intracranial pressure (ICP), presumably secondary to increased edema following LAK cell injection. The median time for tumor recurrence was 22 months; radiographic follow-up in the postoperative period revealed local recurrence or progression in only 12 of the patients. An additional 10 patients died without evidence of tumor recurrence or with tumor at distant sites. Only two patients in the series were reportedly alive and free of tumor recurrence at time of follow-up. Postmortem studies revealed histological evidence of extensive necrosis, reactive astrocytes, and glioma cells at the site of LAK injection. The authors concluded that this therapy offered no advantage over conventional treatment due to the infiltrative nature of these lesions.

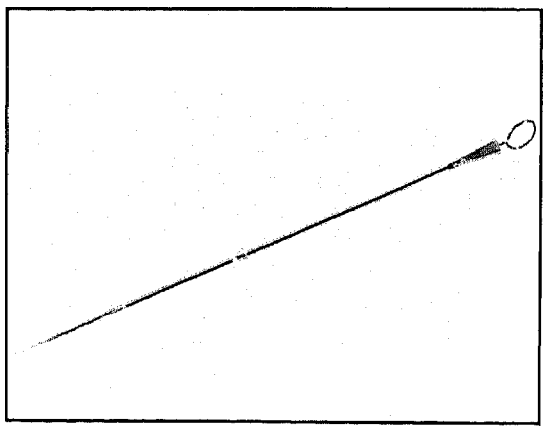

Figure 5. The current methodology for instillation of adoptive immunotherapeutic agents at our institution. Specially designed silicone catheters with holes at variable lengths along the shaft enable delivery of predetermined aliquots of reactants to the tumor bed and margins (A). The flanges are compatible with standard syringe systems for injection. A collar sutured to the scalp permits secure fixation of the catheter. These catheters are stereotactically placed utilizing either CT or MRI guidance in an array to facilitate delivery of LAK cells or lymphokines throughout the viable tumor and tumor margins.

Experience at our institution with LAK cell implants has facilitated the development of specially designed catheters. These are used in stereotactic array implantation at predetermined points at or beyond the tumor margins to incorporate the leading edge. This method takes advantage of contemporary imaging to deliver immunologically active elements to a structurally defined area rather than relying on conceptually blind open methods for component delivery. The catheters are flanged and are compatible with standard luer-lock syringe systems to allow repeat injections of LAK cells or interleukin following surgical placement (Figure 5). The catheters are placed using image-directed stereotaxy (CT or MRI) under local anesthesia; this enables precise localization based on radiographic imaging not obtainable via open craniotomy, with the additional benefit of catheters placed at the margins delivering the LAK cells to potential regions of infiltration. Injection of immunotherapeutic agents is performed at regular intervals postoperatively at the bedside. The catheters are then removed aseptically at the bedside after the treatment course is complete. This method has proven simple and safe, is associated with minimal patient morbidity, and obviates the attendant risks of general anesthesia. To date, use of activated cells or cell products (lymphokines) has not been shown to significantly alter survival in patients with malignant gliomas.

Restorative Immunotherapy

Immunorestoration of depressed cellular immunity may be undertaken with agents such as thymic hormones,[18] of which a number of fractions have been isolated. These agents have the capacity to restore depressed T-cell subpopulations to normal levels, both from a quantitative and qualitative standpoint. Other substances, such as Cytoxan, have the ability to inhibit specifically sup-

high-grade oligodendroglioma. All patients had intraparenchymal injections of the LAK cells with rIL-2 into the tissue surrounding the tumor cavity. For a period of three days, all patients underwent subsequent daily injections of rIL-2 into the tumor cavity via an Ommaya reservoir or through the scalp flap. They then received an additional course of intracavitary LAK and rIL-2 10 days postoperatively. Patients all experienced increased intracranial pressure (ICP), presumably secondary to increased edema following LAK cell injection. The median time for tumor recurrence was 22 months; radiographic follow-up in the postoperative period revealed local recurrence or progression in only 12 of the patients. An additional 10 patients died without evidence of tumor recurrence or with tumor at distant sites. Only two patients in the series were reportedly alive and free of tumor recurrence at time of follow-up. Postmortem studies revealed histological evidence of extensive necrosis, reactive astrocytes, and glioma cells at the site of LAK injection. The authors concluded that this therapy offered no advantage over conventional treatment due to the infiltrative nature of these lesions.

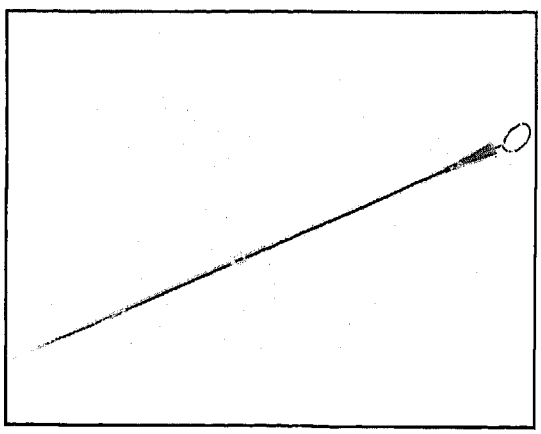

Figure 5. The current methodology for instillation of adoptive immunotherapeutic agents at our institution. Specially designed silicone catheters with holes at variable lengths along the shaft enable delivery of predetermined aliquots of reactants to the tumor bed and margins (A). The flanges are compatible with standard syringe systems for injection. A collar sutured to the scalp permits secure fixation of the catheter. These catheters are stereotactically placed utilizing either CT or MRI guidance in an array to facilitate delivery of LAK cells or lymphokines throughout the viable tumor and tumor margins.

Experience at our institution with LAK cell implants has facilitated the development of specially designed catheters. These are used in stereotactic array implantation at predetermined points at or beyond the tumor margins to incorporate the leading edge. This method takes advantage of contemporary imaging to deliver immunologically active elements to a structurally defined area rather than relying on conceptually blind open methods for component delivery. The catheters are flanged and are compatible with standard luer-lock syringe systems to allow repeat injections of LAK cells or interleukin following surgical placement (Figure 5). The catheters are placed using image-directed stereotaxy (CT or MRI) under local anesthesia; this enables precise localization based on radiographic imaging not obtainable via open craniotomy, with the additional benefit of catheters placed at the margins delivering the LAK cells to potential regions of infiltration. Injection of immunotherapeutic agents is performed at regular intervals postoperatively at the bedside. The catheters are then removed aseptically at the bedside after the treatment course is complete. This method has proven simple and safe, is associated with minimal patient morbidity, and obviates the attendant risks of general anesthesia. To date, use of activated cells or cell products (lymphokines) has not been shown to significantly alter survival in patients with malignant gliomas.

Restorative Immunotherapy

Immunorestoration of depressed cellular immunity may be undertaken with agents such as thymic hormones,[18] of which a number of fractions have been isolated. These agents have the capacity to restore depressed T-cell subpopulations to normal levels, both from a quantitative and qualitative standpoint. Other substances, such as Cytoxan, have the ability to inhibit specifically sup-

pressor cells, thereby enhancing overall immune system activity. To date there is no substantial clinical evidence for the efficacy of this therapy in the specific treatment of glioma.

Passive Immunotherapy

Identification of a tumor-specific antigen would allow the use of hybridoma technology to develop antitumor antibodies to mediate antibody-dependent, cell-mediated killing of selected glioma cells. This mode of treatment has been shown to be effective in rodents against dispersed tumors, particularly leukemia. It does carry the inherent danger of the creation of blocking factors (antigen-antibody complexes) that may occur in vivo, with consequent enhancement of tumor growth. In addition to ADCC, conjugation of MAB with cytotoxic chemicals or radionuclides may offer another potential avenue by which to direct a specific cytotoxic response against glioma cells, should specific antigens be identified (Table 2).

Future Perspectives and Approaches to Immunotherapy

If immunotherapy is to be used to its maximum potential in treating malignant disease, clarification of fundamental issues related to immune mechanisms in glioma-bearing patients must be realized. Historically, the proven beneficial effects of immunotherapy in the treatment or prevention of infectious disease have been based on the development of a specific immunity. This should be an active area of future therapeutic exploration, and the definition of tumor-specific antigens is central to the task. By analogy with other mammalian models and evidence from previously discussed work, it is likely that tumor-specific or highly tumor-associated antigens do exist. Monoclonal antibodies produced by hybridoma methodology are facilitating the search.

Another area of critical importance is identification of the humoral and suppressor elements that potentially thwart endogenous and immunotherapeutic limitation of tumoral growth. Elimination of these substances is a goal for investigators. If molecules produced by the tumor have inhibitory influences on LAK cell activity, monoclonal antibodies developed to neutralize this suppression may potentially be used (Table 3).[35]

TABLE 2
Potential Applications of MAB for Gliomas*

Diagnosis:
- Immunopathology
- Cytopathology: CSF

Therapy:
- **MAB Cytotoxicity:**
  - Complement or Cell-Mediated
- **Inhibition of Receptors for Growth Factors**
- **Conjugation with Cytotoxic Agents:**
  - Drug
  - Toxin
  - Radionuclide

*Adapted from de Tribolet et al.[21]

TABLE 3
Current and Potential Immunotherapeutic Methods

Active Specific
- e.g. autografts, "tumor vaccines"

Active Nonspecific
- e.g. BCG inoculations
  - Low-dose interferons

Adoptive
- e.g. autologous lymphocyte infusions
  - LAK cell implantations

Restorative
- e.g. immunorestorative agents
  - (thymic fractions)

Passive
- e.g. MAB

Combination
- e.g. LAK with MAB
  - Lymphokine combinations
    - (IL-2 and interferon)

The presence of the NK cells in gliomas, with their inherent antitumor capacity, and the ability to enhance NK cytotoxicity with interferons is a potential future experimental avenue for immunotherapy.[9]

The use of newly isolated lymphokines, alone or in combination with established lymphokines, is a promising area of future research. Interleukin-4 has the ability to generate LAK cells and is synergistic with IL-2 in the generation of LAK cells in mice.[50] IL-4 also aids in T-cell growth and differentiation exclusive of LAK cell generation. Other combinations of lymphokines, such as tumor necrosis factor (TNF) and gamma-interferon, exert synergistic in vivo antitumor effects in mice.[15] To date none of these combinations has been attempted in the treatment of human glioma; controlled trials have yet to establish their efficacy in vivo.

Local immunotherapy via intraarterial infusion may preclude the necessity of craniotomy for instillation of immunotherapeutic agents. Interferons or TNF may be injected via carotid artery to achieve high local concentrations in the tumor vasculature, while minimizing systemic toxicity.

The combination of different modes of immunotherapy holds promise for future trials; for example the use of adoptive immunotherapy such as LAK cells with cytolytic monoclonal antibodies may be a future consideration. It is conceivable that with the added factor of ADCC a more favorable response in tumor reduction may be achieved. LAK cells could be generated and infused intratumorally in combination with monoclonal antibodies and cytokines such as TNF or interferon-γ, which synergize with interleukin-2 in maintaining the cell-mediated cytotoxicity and ADCC activity of the infused cells.[1,2]

"Biological response modification" or "Biomodulation" is the central theme to the approach to immunotherapy. It defines the direction to be followed in future immunotherapeutic practice.

References

1. Agah R, Malloy B, Sherrod A, Bean P, Girgis E, Mazumder A. Therapy of disseminated natural killer-resistant tumor by the synergistic effects of recombinant interleukin-2 and tumor necrosis factor. *J Biol Response Mod.* 1988;7:140–151.
2. Agah R, Malloy B, Sherrod A, Mazumder A. Successful therapy of natural killer-resistant pulmonary metastases by the synergism of γ-interferon with tumor necrosis factor and interleukin-2 in mice. *Cancer Res.* 1988;48:2245–2248.
3. Apuzzo MLJ, Mitchell MS. Immunological aspects of intrinsic glial tumors. *J Neurosurg.* 1981;55:1–18.
4. Band PR, Jao-King C, Urtasun RC, Haraphongse M. Phase I study of *Corynebacterium parvum* in patients with solid tumors. *Cancer Chemother Rep.* 1975;59:1139–1145.
5. Bertrand I, Mannen H. Étude des réactions vasculaires dans les astrocytomes. *Rev Neurol.* 1960;102:3–19.
6. Bloom HJG, Peckham MJ, Richardson AE, Alexander PA, Payne PM. Glioblastoma multiforme: a controlled trial to assess the value of specific active immunotherapy in patients treated by radical surgery and radiotherapy. *Br J Cancer.* 1973;27:253–267.
7. Bloom WH, Carstairs KC, Crompton MR, McKissock W. Autologous glioma transplantation. *Lancet.* 1960;2:77–78.
8. Bosnes V, Hirschberg H. Comparison of *in vitro* glioma cell cytotoxicity of LAK cells from glioma patients and healthy subjects. *J Neurosurg.* 1988;69:234–238.
9. Braun DP, Penn RD, Harris JE. Regulation of natural killer cell function by glass-adherent cells in patients with primary intracranial malignancies. *Neurosurgery.* 1984;15:29–33.
10. Brooks WH, Caldwell HD, Mortara RH. Immune responses in patients with gliomas. *Surg Neurol.* 1974;2:419–423.
11. Brooks WH, Latta RB, Mahaley MS, Roszman TL, Dudka L, Skaggs C. Immunobiology of primary intracranial tumors, V: correlation of a lymphocyte index and clinical status. *J Neurosurg.* 1981;54:331–337.
12. Brooks WH, Markesbery WR, Gupta GD. Relationship of lymphocyte invasion and survival of brain tumor patients. *Ann Neurol.* 1978;4:219–224.
13. Brooks WH, Netsky MG, Normansell DE, Horwitz DA. Depressed cell-mediated immunity in patients with primary intracranial tumors: characterization of a humoral immunosuppressive factor. *J Exp Med.* 1972;136:1631–1647.
14. Brooks WH, Roszman TL, Rogers AS. Impairment of rosette-forming T lymphocytes in patients with intracranial tumors. *Cancer.* 1976;37:1869–1873.
15. Brouckaert PGG, Leroux-Roels GG, Guisez Y, Tavernier J, Fiers W. *In vivo* anti-tumor activity of recombinant human and murine TNF, alone and in combination with murine IFN-γ, on a syngeneic murine melanoma. *Int J Cancer.* 1986;38:763–769.
16. Cantrell DA, Smith KA. The interleukin-2 T-cell system: a new cell growth model. *Science.* 1984;224:1312–1316.

17. Coakham HB. Immunology of human brain tumors. *Eur J Cancer Clin Oncol.* 1984;20:145–149.
18. Cohen MH, Chretien PB, Ihde DC, Fossieck BE Jr, Makuch R, Bunn PA Jr. Thymosin fraction V and intensive combination chemotherapy: prolonging the survival of patients with small-cell lung cancer. *JAMA.* 1979;241:1813–1815.
19. de Martin R, Haendler B, Hofer-Warbinek R, Gaugitsch H, Wrann M, Schlüsener H, et al. Complementary DNA for human glioblastoma-derived T cell suppressor factor, a novel member of the transforming growth factor-β gene family. *EMBO J.* 1987;6:3873–3877.
20. de Muralt B, de Tribolet N, Diserens AC, Carrel S, Mach JP. Reactivity of antiglioma monoclonal antibodies for a large panel of cultured gliomas and other neuroectoderm derived tumors. *Anticancer Res.* 1983;3:1–6.
21. de Tribolet N, Frank E, Mach JP. Monoclonal antibodies: their application in the diagnosis and management of CNS tumors. *Clin Neurosurg.* 1988;34:446–456.
22. Duff TA, Borden E, Bay J, Piepmeier J, Sielaff K. Phase II trial of interferon-β for treatment of recurrent glioblastoma multiforme. *J Neurosurg.* 1986;64:408–413.
23. Eardley DD, Hugenberger J, McVay-Bodreau L, Shen FW, Gershon RK, Cantor H. Immunoregulatory circuits among T-cell sets, I: T-helper cells induce other T-cell sets to exert feedback inhibition. *J Exp Med.* 1978;147:1108–1115.
24. Elliott LH, Brooks WH, Roszman TL. Cytokinetic basis for the impaired activation of lymphocytes from patients with primary intracranial tumors. *J Immunol.* 1984;132:1208–1215.
25. Elliott LH, Brooks WH, Roszman TL. Activation of immunoregulatory lymphocytes obtained from patients with malignant gliomas. *J Neurosurg.* 1987;67:231–236.
26. Eremin O, Coombs RRA, Plumb D, Ashby J. Characterization of the human natural killer (NK) cell in blood and lymphoid organs. *Int J Cancer.* 1978;21:42–50.
27. Gately CL, Muul LM, Greenwood MA, Papazoglou S, Dick SJ, Kornblith PL, et al. *In vitro* studies on the cell-mediated immune response to human brain tumors, II: leukocyte-induced coats of glycosaminoglycan increase the resistance of glioma cells to cellular immune attack. *J Immunol.* 1984;133:3387–3395.
28. Gerdes J, Schwab U, Lemke H, Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int J Cancer.* 1983;31:13–20.
29. Greene HSN. Heterotransplantation of tumors. *Ann NY Acad Sci.* 1957;69:818–829.
30. Grimm EA, Mazumder A, Zhang HZ, Rosenberg SA. Lymphokine-activated killer cell phenomenon: lysis of natural killer-resistant fresh solid tumor cells by interleukin-2-activated autologous human peripheral blood lymphocytes. *J Exp Med.* 1982;155:1823–1841.
31. Habel K, Belcher JH. Immunologically privileged sites in studies of polyoma tumor antigens. *Proc Soc Exp Biol Med.* 1963;113:148–152.
32. Jacobs SK, Wilson DJ, Kornblith PL, Grimm EA. *In vitro* killing of human glioblastoma by interleukin-2-activated autologous lymphocytes. *J Neurosurg.* 1986;64:114–117.
33. Jacobs SK, Wilson DJ, Kornblith PL, Grimm EA. Interleukin-2 and autologous lymphokine-activated killer cells in the treatment of malignant glioma: preliminary report. *J Neurosurg.* 1986;64:743–749.
34. Kedar E, Rezai AR, Giorgi JV, Gale RP, Champlin RE, Mitsuyasu RT, et al. Immunomodulating effects in vitro of interleukin-2 and interferon-γ on human blood and bone marrow mononuclear cells. *Nat Immun Cell Growth Regul.* 1988;7:13–30.
35. Kim B, Warnaker P, Iverson M, Imbembo AL. Immunotherapy of pulmonary metastases using monoclonal antibody to T-cell suppressor factor and interleukin-2. *Arch Surg.* 1987;122:1455–1459.
36. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature.* 1975;256:495–497.
37. Korosue K, Takeshita I, Mannoji H, Fukui M, Interferon effects on multiplication, cytoplasmic protein and GFAP content, and morphology in human glioma cells. *J Neurooncol.* 1983;1:69–76.
38. Levy NL. Cell-mediated cytotoxicity and serum-mediated blocking: evidence that their associated determinants on human tumor cells are different. *J Immunol.* 1978;121:916–922.
39. Lotze MT, Matory YL, Ettinghausen SE, Rayner AA, Sharrow SO, Seipp CAY, et al. In vivo administration of purified human interleukin 2, II: half life, immunologic effects, and expansion of peripheral lymphoid cells in vivo with recombinant IL 2. *J Immunol.* 1985;135:2865–2875.
40. Lundblad D, Lundgren E. Block of glioma cell line in S by interferon. *Int J Cancer.* 1981;27:749–754.
41. Mahaley MS Jr, Bertsch L, Cush S, Gillespie GY. Systemic gamma-interferon therapy for recurrent gliomas. *J Neurosurg.* 1988;69:826–829.
42. Mahaley MS Jr, Urso MB, Whaley RA, Blue M, Williams T, Guasperi A, et al. Immunobiology of primary intracranial tumors, X: therapeutic efficacy of interferon in the treatment of recurrent gliomas. *J Neurosurg* 1985;63:719–725.
43. Mazumder A, Rosenberg SA. Successful immunotherapy of natural killer-resistant established pulmonary melanoma metastases by the intravenous adoptive transfer of syngeneic lymphocytes activated *in vitro* by interleukin 2. *J Exp Med.* 1984;159:495–507.
44. McComb RD, Bigner DD. The biology of malignant gliomas: a comprehensive survey. *Clin Neuropathol.* 1984;3:93–106.
45. Medawar PB. Immunity to homologous grafted skin, III: the fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. *Br J Exp Pathol.* 1948;29:58–69.
46. Merchant RE, McVicar DW, Merchant LH, Young HF. Long term follow up of patient with recurrent malignant glioma treated with intraparenchymal injections of Lymphokine-activated killer cells and recombinant interleukin-2. Presented at plenary session, American Association of Neurological Surgeons; April 1–4, 1989; Washington, D.C.
47. Mitchell MS, Kempf RA, Harel W, Shau H, Boswell WD, Lind S, et al. Effectiveness and toler-

ability of low-dose cyclophosphamide and low-dose intravenous interleukin-2 in disseminated melanoma. *J Clin Oncol.* 1988;6:409–424.
48. Mitchell MS, Murahata RI. Modulation of immunity by bacillus Calmette-Guérin (BCG). *Pharmacol Ther.* 1979;4:329–353.
49. Morantz RA, Shain W, Cravioto H. Immune surveillance and tumors of the nervous system. *J Neurosurg.* 1978;49:84–92.
50. Mulé JJ, Smith CA, Rosenberg SA. Interleukin 4 (B cell stimulatory factor 1) can mediate the induction of lymphokine-activated killer cell activity directed against fresh tumor cells. *J Exp Med.* 1987;166:792–797.
51. Murphy JB, Sturm E. Conditions determining the transplantability of tissues in the brain. *J Exp Med.* 1923;38:183–197.
52. Nagai M, Arai T. Clinical effect of interferon in malignant brain tumours. *Neurosurg Rev.* 1984;7:55–84.
53. Nederman T, Benediktsson G. Effects of interferon on the growth rate and radiation sensitivity of cultured, human glioma cells. *Acta Radiol Oncol.* 1982;21:231–234.
54. Oehler JR, Herberman RB, Holden HT: Modulation of Immunity by Macrophages. *J Pharmacol Exp Ther.* 1978;20:551–593.
55. Palma L, Di Lorenzo N, Guidetti B. Lymphocytic infiltrates in primary glioblastomas and recidivous gliomas: incidence, fate, and relevance to prognosis in 228 operated cases. *J Neurosurg.* 1978;49:854–861.
56. Parrillo JE, Fauci AS. Mechanisms of corticosteriod action on lymphocyte subpopulations, III: differential effects of dexamethasone administration on subpopulations of effector cells mediating cellular cytotoxicity in man. *Clin Exp Immunol.* 1978;31:116–125.
57. Paterson PY. Transfer of allergic encephalomyelitis in rats by means of lymph node cells. *J Exp Med.* 1960;111:119–136.
58. Quesenberry P, Levitt L: Hematopoietic stem cells. *N Engl J Med.* 1979;301:755–761.
59. Reinherz EL, Schlossman SF. Regulation of the immune response—inducer and suppressor T-lymphocyte subsets in human beings. *N Engl J Med.* 1980;303:370–373.
60. Ridley A, Cavanagh JB. Lymphocytic infiltration in gliomas: evidence of possible host resistance. *Brain.* 1971;94:117–124.
61. Rosenberg SA. Immunotherapy of cancer using interleukin-2: current status and future prospects. *Immunol Today.* 1988;9:58–62.
62. Roszman TL, Brooks WH, Elliott LH. Immunobiology of primary intracranial tumors, VI: suppressor cell function and lectin-binding lymphocyte subpopulations in patients with cerebral tumors. *Cancer.* 1982;50:1273–1279.
63. Roszman TL, Brooks WH, Elliot LH. Inhibition of lymphocyte responsiveness by a glial tumor cell-derived suppressive factor. *J Neurosurg.* 1987;67:874–879.
64. Roszman TL, Brooks WH, Steele C, Elliott LH. Pokeweed mitogen-induced immunoglobulin secretion by peripheral blood lymphocytes from patients with primary intracranial tumors: characterization of T helper and B cell function. *J Immunol.* 1985;134:1545–1550.
65. Shau H, Gray JD, Mitchell MS. Studies on the relationship of human natural killer cells and lymphokine-activated killer cells with lysosomal staining and analysis of surface marker phenotypes. *Cell Immunol.* 1988;115:13–23.
66. Shuttleworth EC Jr. Barrier phenomena in brain tumors. *Prog Exp Tumor Res.* 1972;17:279–290.
67. Smith RT. Tumor-specific immune mechanisms. *N Engl J Med.* 1968;278:1207–1214, 1268–1275, 1326–1331.
68. Takakura K, Miki Y, Kubo O, Ogawa N, Matsutani M, Sano K. Adjuvant immunotherapy for malignant brain tumors. *Jpn J Clin Oncol.* 1972;12:109–120.
69. Takeuchi J, Barnard RO. Perivascular lymphocytic cuffing in astrocytomas. *Acta Neuropathol.* 1976;35:265–271.
70. Uede T, Kohda H, Ibayashi Y, Osawa H, Diamantstein T, Kikuchi K. Functional analysis of mononuclear cells infiltrating into tumors, II: differential ability of mononuclear cells obtained from various tisues to produce helper factors that are involved in the generation of cytotoxic cells. *J Immunol.* 1985;135:3243–3251.
71. Vaquero J, Coca S, Oya S, Martinez R, Ramero J, Salazar FG. Presence and significance of NK cells in glioblastomas. *J Neurosurg.* 1989;70:728–731.
72. von Hanwehr RI, Hofman FM, Taylor CR, Apuzzo MLJ. Mononuclear lymphoid populations infiltrating the microenvironment of primary CNS tumors: characterization of cell subsets with monoclonal antibodies. *J Neurosurg.* 1984;60:1138–1147.
73. Wakabayashi T, Yoshida J, Seo H, Kazo K, Murata Y, Matsui N, et al. Characterization of neuroectodermal antigen by a monoclonal antibody and its application in CSF diagnosis of human glioma. *J Neurosurg.* 1988;68:449–455.
74. Walker MD, Alexander E Jr, Hunt HE, MacCarty CS, Mahaley MS Jr, Mealy J Jr, et al. Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas: a cooperative clinical trial. *J Neurosurg.* 1978;49:333–343.
75. Williams WJ, Beutler E, Erslev AJ, Lichtman MA, eds. *Hematology.* 3 rd ed. New York, NY: McGraw Hill; 1983.
76. Wrann M, Bodmer S, de Martin R, Siepl C, Hofer-Warbinek R, Frei K, et al. T cell suppressor factor from human glioblastoma cells is a 12.5-kd protein closely related to transforming growth factor-β. *EMBO J.* 1987;6:1633–1636.
77. Yamaki T, Uede T, Shijubo N, Kikuchi K. Functional analysis of mononuclear cells infiltrating into tumors, III: soluble factors involved in the regulation of T lymphocyte infiltration into tumors. *J Immunol.* 1988;140:4388–4396.
78. Yoshida J, Kageyama N, Ueda R, Takahashi T. Human glioma associated and related antigens, identified by monoclonal antibodies. *Neurosurg Rev.* 1984;7:23–28.
79. Yoshida J, Wakabayashi T, Kito A, Kageyama N, Murata Y, Seo H. Clinical application of monoclonal antibodies against glioma-associated antigens. *Prog Exp Tumor Res.* 1987;30:44–56.
80. Yoshida S, Takai N, Tanaka R. Functional analysis

of interleukin-2 in immune surveillance against brain tumors. *Neurosurgery*. 1987;21:627–630.
81. Young HF, Kaplan A, Regelson W. Immunotherapy with autologous white cell infusions "lymphocytes" in the treatment of recurrent glioblastoma multiforme: a preliminary report. *Cancer*. 1977;40:1037–1044.
82. Yron I, Wood TA Jr, Spiess PJ, Rosenberg SA. *In vitro* growth of murine T cells, V: the isolation and growth of lymphoid cells infiltrating syngeneic solid tumors. *J Immunol*. 1980;125:238–245.
83. Zuber P, Hamou M-F, de Tribolet N. Identification of proliferating cells in human gliomas using the monoclonal antibody Ki-67. *Neurosurgery*. 1988;22:364–368.